CHAPTER 6

STUDIES ON THE LYMPHOCYTOPOIESIS IN THE LIVER AND SPLEEN OF THE ROSY PASTOR AND CERTAIN OTHER BIRDS

Active haematopoiesis has not been reported to occur in the livers of adult birds and mammals. But, during the embryonic period, the livers of both these groups were found to produce numerous blood cells. Though the liver carries out this function in the embryonic period in majority of the birds, the chick embryo liver was not found to be a haematopoietic organ (Dantschakoff,1908). In Sturnus vulgaris, haematopoiesis starts in the liver during the late embryonic period and continues even after hatching (Sandreuter,1951).

Although, haematopoietic activities of the liver are restricted to the embryonic period, it is incorrect to believe that the adult liver looses its capacity to produce blood cells. As a part of the reticuloendothelial system, the liver takes up haematopoiesis whenever the need arises, either due to bone marrow malfunction or when its production becomes insufficient to meet the demand. Thus, in pathological conditions, such as depressed bone marrow function, the liver becomes a site of blood cell production (Popper and Schaffner,1957). It was also observed that, when spleen and bone marrow were damaged or when incurred a heavy loss of blood, haematopoiesis was seen in the liver (Bessis,1956; Popper and Schaffner,1957). Similarly, in pathological conditions of the liver itself, haematopoiesis takes place therein (Black-Schaffer and Stoddard,1953; Peace, 1953).

Recently, George and Naik (1963) observed a constant occurrence of haematopoietic nodules in the liver of the migratory starling (Sturnus roseus). These nodules were reported to be lymphocytopoietic in nature.

The present investigation was undertaken with a view to find out whether such nodules were present in the liver of other birds too, and if so, to elucidate their significance.

MATERIALS AND METHODS

Various birds such as Rosy Pastor (Sturnus roseus), Brahminy Myna (Sturnus pagodarum), Common Myna (Acridotherus tristis), Bank Myna (A. ginginianus), Pigeon (Columba livia) and Domestic Fowl (Gallus domesticus) were utilized for this study.

For histological studies the liver pieces were fixed in Bouin's fluid and the paraffin sections were stained with haematoxylin-eosin. The spleen was also processed in the same way. To demonstrate fat histochemically, pieces of liver and spleen were fixed in calcium formol solution. Frozen sections of the liver and spleen were stained with Sudan Black B, Fettrot 7B, Oil Red O and Nile blue sulphate as described by Pearse (1960).

The measurements of diameter ~~etc.~~, were carried out on frozen sections only. The number of nodules per unit area of liver and spleen was determined by counting them in a 3.5 X objective lens field.

RESULTS

Of all the birds studied only the Pigeon, besides Rosy Pastor, contained constantly appearing nodules in the liver. These nodules were present in the Pigeon liver throughout the year.

The haematopoietic nodules found in the Pigeon liver in large numbers were of varying sizes and shapes. Some of these were circular and lined with connective tissue sheath, while others were irregularly shaped (Figs. 1 & 2). They contained mostly lymphocytes, with a prominent nucleus having the basic nuclear units (chromatin lumps) as described by Engelbert (1956), while the basophilic cytoplasm formed only a thin layer around the nucleus. A majority of the lymphocytes were of the smaller type and those in the centre of the nodule showed mitotic activity. As in the case of *Sturnus roseus* (George and Naik, 1963), the haematopoietic nodules in the pigeon liver were also seen attached to or associated with blood vessels, hence the portal spaces were found to lodge a large number of them. There were no indications of erythropoiesis in these nodules, nevertheless, mature erythrocytes were seen in the near viscinity.

The number of nodules per unit area was found to be a variable factor in different individuals. A close association between the number of nodules and the amount of fat (detected by staining intensity) in the liver was noticed (Tables I & II). A similar correlation with fat deposition in the adipose tissue

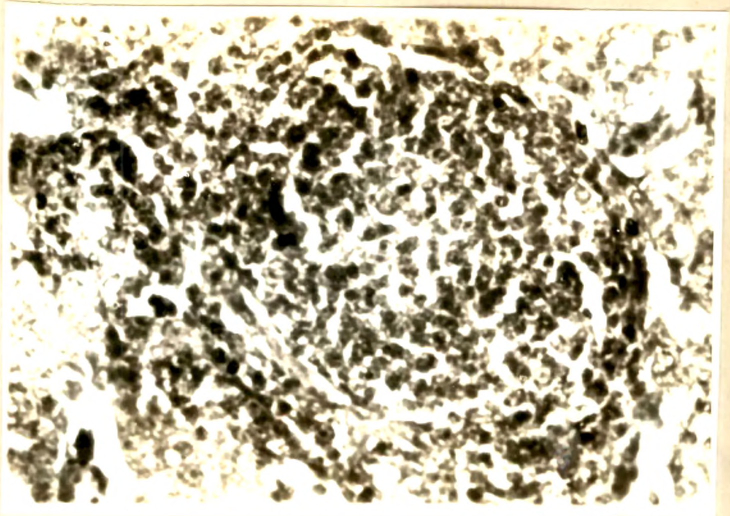

Fig.1. Microphotograph of the section of Pigeon liver showing the round nodules. Cells in the centre are in mitotic stage. Stained with haematoxylin-eosin. 400x.

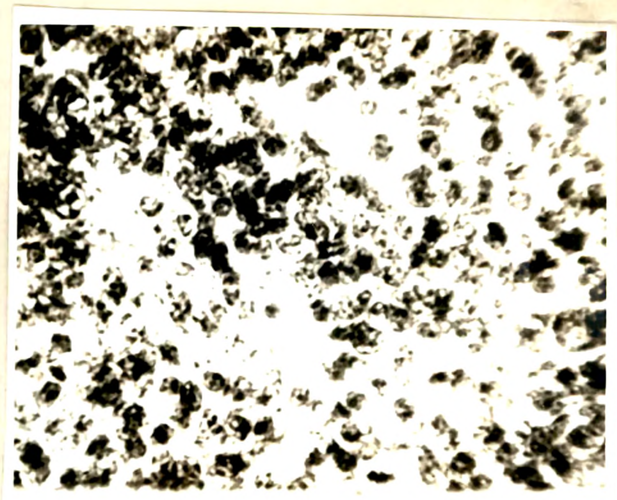

Fig.2. Higher magnification of the nodular region. Majority of the cells are small lymphocytes with basic nuclear units (net work of chromatin) with indistinguishable cytoplasm. Haematoxylin-eosin stained. 630x.

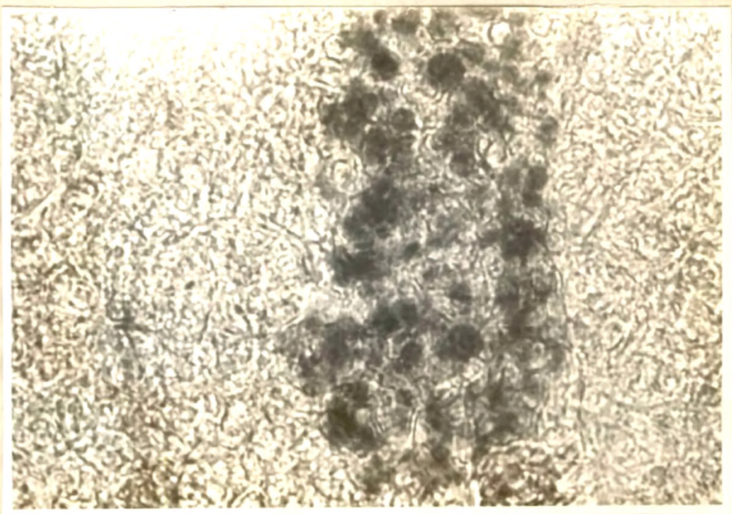

Fig.3A. Microphotograph of the sections of Pigeon liver showing the presence of neutral fat in some nodular cells. Stained with Fettrot 7B. 300x

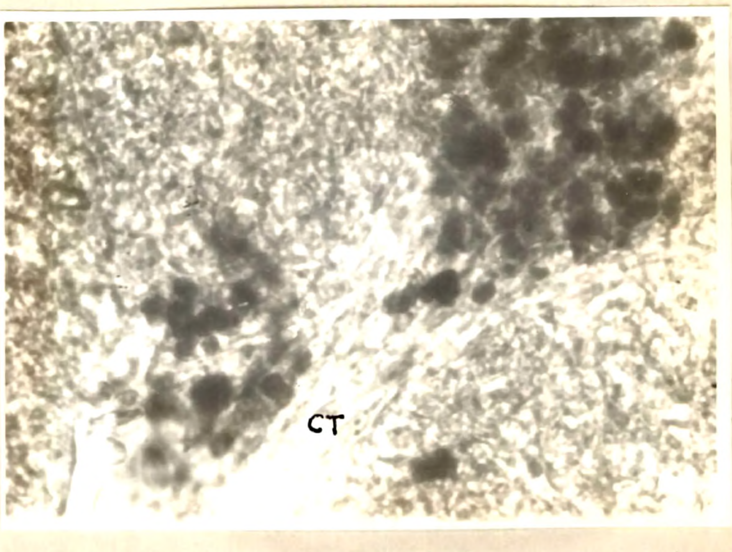

Fig.3B. Microphotograph of another nodular region showing the presence of neutral fat in some cells of the nodule. Note the proximity of the nodules to the connective tissue (CT) in the portal spaces. 300x.

TABLE I

Number and diameter of haematopoietic nodules in the liver and the size of spleen and its nodules and their relationship with the fat contents of liver and adipose tissue of wild pigeons.

Weight of the body Gram	Average nodule count per 3.5X objective lens field in the liver	Total No: of nodules in five fields	Diameter of big round nodules µ	diameter of the spleen mm	Diameter of nodules in the spleen µ	Fat content in the liver	Fat content in the adipose tissue
282	6	25	225	5	255	+++	+++
265	4	18	352	5	225	++	++
270	4	16	337	4.5	195	++	++
254	4	14	213	5	165	++	++
190	3	16	300	5	168	+	+
185	3	16	185	4	171	+	+
178	3	12	172	3	172	+	+
173	3	8	163	3	153	+	+
181	2	9	165	3.5	157	+	+
173	2	9	157	3	152	+	+
175	1	5	...	2	142	+	+
180	1	5	...	2	129	+	-

+ very little fat, ++ fairly good amount of fat, +++ very good amount of fat, - nil.

TABLE II

Number and diameter of haematopoietic nodules in the lixer and the size of spleen and its nodules and their relationship with the fat contents in the liver and adipose tissue of domestic pigeons.

Weight of the body Gram	Average nodule count per 3.5X objective lens field in the liver	Total No: of nodules in five fields	Diameter of big round nodules μ	Diameter of the spleen mm	Diameter of nodules in the spleen μ	Fat content in the liver	Fat content in the adipose tissue
394	16	82	255	12	225	++++	++++
390	8	40	180	8	360	+++	++++
348	8	43	247	7	240	+++	++++
388	7	38	188	6	265	+++	++++
381	7	37	155	7	255	+++	++++
375	5	23	120	6	235	+++	++++
340	4	19	163	5	220	++	+++
338	4	18	148	4	215	++	+++
320	3	12	215	4	225	+++	++
320	2	6	150	4	210	+	+
308	1	5	90	3	165	+	+

+ very little fat, ++ fairly good amount of fat, +++ very good amount of fat, ++++ heavily filled.

TABLE III

Weight of the spleen of the Rosy Pastors during the post- and pre- migratory periods and the corresponding fat content in the liver and the size of the adipose tissue.

Date	Weight of the body Grams	Weight of the spleen Grams	Fat content in the liver g/100g wet tissue	Size of the adipose tissue
AUGUST 1 -31 (post migratory period)	78*	0.14*	10*	+++
MARCH 1 -15	65	0.061	12	+
16-30	78	0.066	14	+
APRIL 1 - 7	78	0.091	16	++
8 -15	83	0.103	..	+++
16-23	85	0.148	17	++++
24-31	92	0.276	24	++++

* Average of 10 birds

+ adipose tissue only in the abdominal region

++ seen some what all over the body in the developing state

+++ almost filled to the capacity covering greater areas

++++ heavily loaded all over the body

and the number of nodules in the liver was also seen (Table I and II). The wild and domestic pigeons were treated separatly since the domestic ones were found to be fatter and heavier than the former. This is due to the fact that the domestic ones are mostly inactive and are provided with an ample supply of food. On the other hand, the wild ones, which are very active show less fat in their adipose tissue and liver. In a comparison, the nodular count and the amount of fat in the liver as well as in the adipose tissue in the domestic pigeons (Table II) were found to be higher than in the wild ones (Table I).

The size of the spleen was also found to have some relationship with fat deposition in the Pigeons. In those pigeons which had a heavy deposition of fat in their adipose tissue, an increase in the diameter and number of lymphocytic nodules in the spleen was observed. Similarly too, in Rosy Pastor, the weight of the spleen was found to increase towards the end of April (Table III). In the postmigratory period the weight of the spleen averaged 140 mg. It should be noted that in this period (just after the completion of migration) these birds still contained good amount of fat in the adipose tissue which were unused during their return journey.

The cells in these nodules were found to contain droplets of neutral fat as indicated by the selective staining with Fettrot and Oil red O. The liver also contained only neutral fat (Fig. 3). The presence of neutral fat was not seen in all the cells of nodules but only in a few.

DISCUSSION

In view of the fact that the liver has the capacity to produce different strains of blood cells upon demand, when haematopoietic nodules in considerable numbers were observed in the liver of all the Pigeons examined, their occurrence has to be envisaged as a definite liver function.

The presence of non-hepatic cells in the liver was repprted to be a normal feature in the adult human beings. These cells were of different origins, probably belonging to the lymphocytic and monocytic strains, and were found to accumulate to form nodules. These nodules become larger and more persistent with increasing age (Popper and Schaffner,1957). The lymph follicles thus formed vary in size and pattern (Kettler, 1933; 1940; 1954). These cellular aggregations were not found to accompany any liver disorders (Popper and Schaffner,1957). Such cells could have been provided by the liver itself where they multiply or else are formed as a result of proliferation of some stem cells which have migrated accidentally from extra-hepatic tissues. The latter suggestion is more plausible, since the number of nodules in the human liver was usually so small with no regularity in formation, that they normally escape notice.

However, the presence of large number of nodules in the pigeon liver throughout the year, cannot be taken as an accidental occurrence. Their constant appearance indicates that they have some important physiological functions and were formed as a response to some hitherto unknown stimulus. An increased demand upon the liver could serve as a stimulus, as in the case of spleenctomy whereby the bone marrow of the Pigeon showed the appearance of lymph nodules (Andrew,1959). Lymphocytopoiesis was not observed in the bone marrow of the active fliers, but that of Fowl and Turkey contained lymph nodules (Jordan,1936). This could be to relieve the bone marrow of such function so as to achieve greater efficiency in erythrepoiesis (R.B.C. count is 3.6 million/cmm in Fowl as against 4 to 6 million in passerines: Nice et al.,1935). Normally, the lymphocytes produced by organs such as spleen, wall of the gut, thymus and bursa of Fabricii are sufficient to meet the requirement. Then, this activity showed by the liver could be to meet an additional demand, reasons of which are unknown.

In the light of several experimints by many workers (C.f. Yoffey and Courtice,1956) it is now realized that the lymphocytes are capable of transferming into a wide veriety of cells. It was shown that they are functionally heterogenous (Yoffey,1964b) and by employing phytohaemagglutinin in leucocyte cultures (Hungerford et al.,1959; Nowell,1960), it was observed that small lymphocytes which had previously been thought to be 'inactive', became active and underwent growth and proliferation, (Yoffey,1964b;Johnson and Roberts,1964). Thus, Jordan (1935) had emphasized the importance of lymphocytes in the red cell production in birds, while Jordan and Johnson (1935) reported that in regenerating bone marrow of pigeons, lymphocytes and like cells actively migrated and finally developed into typical haemocytoblasts. In other birds with nodules in the bone marrow itself, the lymphocytes produced therein entered the vascular channels of marrow and developed into both red cells and granulocytes (Jordan,1936). Yoffey and Courtice (1956), Keiser _et al_.(1964) and Bond _et al_. (1964) suggested a similar fate for blood lymphocytes because they could trace the passage of these cells into the bone marrow.

With such an array of evidences, it could be reasoned that a certain number of lymphocytes produced elsewhere in the body, would be taken up by the marrow and utilized for the production of other types of blood cells. This was shown experimentally, whereby removal of 40% bone marrow from the rat was followed by an increase in number of blood lymphocytes (Steinberg,1946; Steinberg and Martin,1964). If this is so, an increased lymphocytic migration into the bone marrow should take place when an elevated erythropoiesis occurs. Thus during secondary hypoxia in guinea pigs, Yoffey (1964a) showed a large increase of lymphocytes in the bone marrow. It might be possible that the production of large number of lymphocytes in the liver was to supplant the spleenic production. In this connection, the fact that, there occurred an increased erythropoiesis in the Rosy Pastor during the premigratory period (Chapter 5) should also be considered. However, such an explanation could be extended only to the case of Rosy Pastor but not to that of Pigeon, where although an equally large number of nodules were seen, an increased erythropoiesis was not observed.

In the Rosy Pastor a fat synthesizing function was ascribed to the haematopoietic nodules of the liver (George and Naik,1963). In the present study also, some neutral fat containing cells were observed in the nodules in the Pigeon liver. This denotes either a <u>de novo</u> synthesis of neutral fat in these cells or its absorption from the surrounding parenchymal cells which abound in fat. It is well known that reticuloendothelial cells are always seen associated with fat, the former were seen to accumulate fat and get converted into fat cells in the bone marrow and adipose tissue (Wasserman, 1926). The transformation of lymphocytes into monocytes and macrophages in certain cases of inflammations have been reported (Trowell,1958). If macrophages of the peritonial cavity could convert themselves into fat cells (Chang,1940) then the transformation of lymphocytes to fat cells via the macrophage stage could be visualized. The presence of neutral fat in the nodular cells (the type of fat is the main form of stored fat in the adipose tissue) as well as a high nodular count when the liver and adipose tissue hold considerable amount of fat, point out that the lymphocytes produced by liver (and spleen) have some role in the metabolism of fat, perhaps by transporting it from liver to depot or by transforming themselves into fat cells. Transformation of blood cells into fat cells is not an uncommon phenomenon. There are so many reports which show such processes in insects. Here the haemocytes get attached to the fat body and later become a fat cell (Lazarenko,1925).

In these insects the fat body is formed or increased in size by the addition of such cells. In other insects where the haemocytes are not added to the fat body, the existing cells divide and multiply at the time of fat deposition. In the case of these birds (Rosy Pastor and Pigeon) also one could suggest that the adipose tissue may be formed or increased in size by the constant addition of cells like lymphocytes, whereas in other birds, if and when such heavy deposition of fat takes place the existing adipose tissue cells enlarge and may divide too.

As described earlier, the lymphocyte is a kind of 'primitive' cell which is capable of differentiating into other cell types, even other than those of blood. They were reported to undergo tranformation into mast cells through some unknown process (Michels, 1938; Asboe-Hansen,1954). The epithelial cells and lymphocytes of the thymus gland are also capable of being converted into mast cells (Csaba et al.,1960). Since the latter are found to contain heparin, histamine and serotonin, the lymphocytes have to take up these substances or else synthesize them in order to become mast cells. That embryonic organs with greater amounts of muccopolysaccharides, were capable of taking up heparin and with increasing age, this function was limited to the lymphatic organs was reported by Csaba and Kapa (1960). Heparin is known to release a fat clearing factor (lipoprotein lipase) from blood vessels (Korn, 1955a; 1955b; 1957; Robinson and French,1960). Therefore, mast cells with their complement of heparin might play a decisive role in the fat metabolism.

Heparin also releases lipoprotein lipase from rat adipose tissue (Korn,1957). Large number of mast cells were reported in the adipose tissue of obese rats (Hellman et al.,1963). This is in accordance with the reported increase of lipase activity in the adipose tissue during fat deposition (Hollenberg,1959). Since, heparin when added to pig adipose tissue preparations (Lynn and Perryman,1960) and to extracts of chicken adipose tissue (Korn and Quigley, 1955; 1957) failed to activate any fat hydrolysing enzymes, it was concluded that it may only function during the deposition of fat. If the fate of lymphocytes is to transform into mast cells, the increased production of the former in the liver and spleen of Rosy Pastor and Pigeon could be also correlated with fat metabolism.

The fact that the weight of the spleen in the Rosy Pastor was found to increase towards the end of April shows that the lymphocyte production was at an increased rate in all the organs that are concerned with its production. The mean weight of spleen during the post migratory period was also very high, but the presence of considerable amount of fat in the adipose tissue and liver (Table III) could vouch for it. In the Pigeon the diameter of the spleen was found to increase along with the increase in fat in the body. The lymph nodules in the spleen also increase in number and size. It then seems possible to suggest that the production of large number of lymphocytes, no matter from where, helps in some way towards the fat deposition in these birds.

In conclusion it may be mentioned that some kind of stimuli, either hormonal or physiological, influenced the lymphocyte production to be at a higher rate. The nature of the stimuli was such or the demand was so great that the liver too started producing these cells as a regular lymphoid tissue. Perhaps, the lymphocytes produced by the liver may be the most versatile, since the lymphocytes produced at different sites have different potentialities and hence destined for some special purposes.